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13. ABSTRACT *(Maximum 200 Words)*

These studies addressed the role of certain factors on the ability to maintain blood pressure (BP), cardiac output (CO) and O_2 delivery (O_2del) during hemorrhage in the conscious goat. We studied the effects of oxygen content of inspired air, the presence of the spleen, roles of arginine vasopressin (AVP), the renin-angiotensin system, and the presence of estrogen. We observed that a controlled hemorrhage at 0.5 ml/kg/min for 30 min conducted in the same goats while exposed to either 11, 21, and 100% FIO_2 , reduced mean arterial BP by approximately 25, 15, and 5 mmHg respectively. Improved maintenance of BP during hyperoxia was achieved by an earlier rise in systemic vascular resistance, and O_2 consumption was similar in all experiments following hemorrhage. Presence of the spleen did not affect the magnitude of drop in BP, O_2del, nor hormonal responses to the hemorrhage. In other experiments, i.v. infusions of AVP in goats breathing 11% FIO_2 increased the arterial O_2 concentration, but the AVP-induced decrease in cardiac output prevented an improvement in O_2del. Neither hypoxia nor the rate of AVP infusion affected whole body AVP clearance. Lastly, we observed that estrogen administration to gonadectomized female or male goats lowered the hypotensive threshold during hemorrhage because of a reduced ability to maintain CO.

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J R. Clay 22 Feb. 2000

PI - Signature Date

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INTRODUCTION

When the tissues of the body receive too little O_2 to maintain adequate function, various reflexes respond causing increased respiration and cardiac output. Depending on the cause of the reduced O_2 the systemic vasculature can constrict or dilate. In response to hypoxia, for instance, cardiac output is increased and systemic vascular resistance is reduced (1). This allows for increased flow of blood to the tissues, improved O_2 delivery, and maintenance of normal blood pressure. In the case of hemorrhage, however, the body is also faced with reduced O_2 at the tissues, but by reflexes mediated primarily via low pressure system baroreceptors, an increased sympathetic tone results leading to vascular constriction and increased heart rate (2). This allows the system to maintain blood pressure with hemorrhages up to about 15% of total blood volume, thereby maintaining perfusion pressure and circulation to select areas of the body. Near this volume of hemorrhage, however, there is a sympathetic inhibition. This appears to initiate the life threatening conditions of decreasing mean arterial blood pressure and heart rate with any further loss of blood.

It can be hypothesized from this background, that if hemorrhage were to occur during hypoxia that there would be a conflict of reflexes, hypoxia causing a vasodilatation and hemorrhage causing a vasoconstriction. In fact, we reported an earlier reduction in blood pressure during a controlled hemorrhage in goats during exposure to hypoxia, 10 % inspired O_2, than when breathing a normoxic gas mixture (3). This was subsequently confirmed in experiments using rats that were hemorrhaged either during acute exposures to hypoxia (12% O_2) or after a chronic, 3-week exposures to hypoxia (4). Only the acute exposure to hypoxia was associated with the decreased ability to maintain blood pressure during hemorrhage. Moreover, the chronic exposures increased the threshold of volume of blood loss required to elicit a fall in blood pressure in both the normoxic and hypoxic states (4,5). The mechanism of the latter is unknown.

It could also be hypothesized that if hypoxia causes a decreased ability to maintain blood pressure, that hyperoxia may reduce or eliminate the reduction in mean arterial blood pressure in response to a modest hemorrhage. However, the vasoconstrictor effects of hyperoxia may adversely affect oxygen delivery to certain areas of the body. Therefore, as one objective of these studies, we investigated and compared the ability to maintenance arterial blood pressure during hemorrhage under conditions of normoxia, hyperoxia and hypoxia. In addition, measurements of systemic vascular resistance, O_2 delivery and O_2 consumption were determined.

A second objective of this proposal was to further investigate the effects of vasopressin on O_2 delivery and O_2 consumption. It has been known for many years that vasopressin can be released by stimulation of chemoreceptors (6). Furthermore, hypoxic stimulation of vasopressin appears very early in development, and has been reported in fetal sheep during the third trimester (7). While there exists strong evidence for a role of vasopressin as causal in the often fatal condition of high altitude cerebral edema, and possibly high altitude pulmonary edema (8), there has been a paucity of information concerning the possible beneficial role of vasopressin in the response to hypoxia. Intuitively, it would seem that such an early, ontogentically, developed response, that is present in many if not all mammals, would have some survival value. In previous studies in our laboratory (9,10) we have shown that exogenous, intravenously administered, vasopressin greatly improves PaO_2, SaO_2, and shunt fraction in the anesthetized, artificially ventilated, new born piglet, especially during hypoxic conditions. This response has

not been confirmed in a conscious, spontaneously breathing animal. The adult goat model presently being used in our laboratory was very suitable for these experiments, because, in conjunction with local anesthesia, the jugular vein is readily accessible introduction of a Swan-Ganz catheter for the necessary measurements. In addition, the multiple infusion rates of vasopressin and subsequent plasma levels of vasopressin achieved allowed an opportunity to further study the whole body clearance of vasopressin. It has been previously shown to be positively correlated with plasma levels of vasopressin in dogs and humans (11). This is the first study to determine the effects of hypoxia on the whole body clearance of vasopressin.

The role of the spleen in animals with contractile spleens, has been thought to be that of a reservoir of blood. Therefore, in studies involving hemorrhage in animals with contractile spleens, the spleen is often removed, presumably so the animal will respond more like a human, because humans do not have contractile spleens (12). The spleen, however, may have other effects. For instance, Horton et al. (13) have reported evidence that dogs with their spleens intact, respond to hemorrhage with greater myocardial contractility than splenectomized dogs with or without augmented infusion of blood to mimic the effects of the spleen. Similar studies have not been done in the goat, nor on the modest rate and magnitude of hemorrhage that we have employed in this conscious standing animal. Therefore, in order to better understand the role of the spleen in our animal model we investigated the cardiovascular responses and the O_2 delivery and consumption and hormonal responses in the intact and splenectomized goat.

The third major objective of the proposal was directed at central control mechanisms that affect the maintenance of blood pressure and cardiac output during hemorrhage. Specifically we attempted to test the hypothesis that central mechanisms that are responsible for the sympathetic withdrawal are mediated either directly or influenced by vasopressin. These experiments were not completed due to technical difficulties and slow progress. Also, since the writing of this proposal other work has been reported that supports our hypothesis, and lessens the necessity to complete these studies as originally conceived. For instance, Budzikowski et al. (14) have reported that blockade of central V1 receptors for vasopressin abolished the hemorrhage-induced bradycardia and hypotension in WKY (Wistar Kyoto) rats, but not in SHR (Spontaneously Hypertensive Rats).

As a result of our slow progress and the recent work of others cited in the previous paragraph, we requested, and were granted permission, to postpone those studies and add a new objective during the last 18 months of the grant. Specifically, we have tested the hypothesis that estrogen will reduce the tolerance to hemorrhage. This hypothesis was based on the previous studies that demonstrated that the presence of estrogen reduced the pressor effects of infused angiotensin in sheep (15), and the pressor responsiveness to vasopressin is reduced in the female rat (16), and also, studies by Crofton et al (17) provided suggestive evidence that hemorrhage in female rats during proestrus (periods of high estrogen) resulted in an earlier onset of hypotension than similarly hemorrhaged female rats during other phases of the estrus cycle or than male rats. These later studies were based on the statistical significance of one time period at 10 minutes after the removal of 10% of the blood volume. The present studies were conducted to further these observations by determining if the effects may be due to gonadal steroids, specifically, estradiol-17β, whether the effects are due to reductions in CO or SVR, and whether the effects of estrogen can be manifest in both male and female animals after gonadectomy.

BODY:

Experimental Methods:

Young adult female and some castrated male goats, weighing about 30-45 kg, were used. This grant involved three protocols that were approved by the Tripler Army Medical Center Institutional Animal Care and Use and Committee. The facility and program are AAALAC approved.

All goats were prepared with an exteriorized carotid arterial loop, as described earlier (3), at least 3 weeks before any experiments. This allows repeated access to the artery for purposes of arterial pressure recordings, heart rate, arterial blood gas and cooximetery measurements, hormone samples, and blood removal for hemorrhage.

On the experiment day, the goats were placed in a stanchion and the areas of the carotid loop and the contralateral jugular vein were shaved and swabbed with antiseptic. The carotid artery was cannulated and the line connected to a pressure transducer and recorder. After local anesthesia, a small skin incision was made over the jugular vein, and an introducer was inserted into the jugular vein, and a Swan-Ganz catheter was inserted through the introducer into the jugular vein and advanced to the pulmonary artery. Location was determined by monitoring the pressures at the tip and at the point used to measure central venous pressure (CVP), while advancing the catheter. With these catheters in place, we measured mean arterial pressure (MABP), and the systolic and diastolic pressures, heart rate, CVP, pulmonary arterial pressure (PAP), pulmonary arterial wedge pressure (PAWP), and cardiac output (CO) was determined by thermodilution.

Infusions were made via the port to the area of the right atrium in the Swan-Ganz catheter, and blood removal was from the arterial or the Swan-Ganz catheter depending upon whether arterial or venous blood was needed. With assessments of O_2 content by cooximetery and blood gas analysis on the arterial and venous sites, and having measured cardiac out put, calculated values of O_2 delivery and O_2 consumption and of systemic vascular resistance and pulmonary arterial resistance were obtained.

In experiments where hypoxic or hyperoxic gas mixtures were administered, either air, air diluted with nitrogen (hypoxia), or 100% O_2 was delivered through a face mask through a one-way valve, from compressed gas cylinders. The fractional inspired O_2 (FIO_2) was constantly monitored and flow meters were adjusted to deliver either 11%, 21%, or 100% O_2 .

Hemorrhage was performed by removal of blood with a peristaltic pump calibrated to remove 0.5 ml/kg/min. The blood was collected in sterile blood donor bags containing citrate. At the conclusion of the experiment the blood was returned to the animals.

Splenectomy was performed via a left paracostal approach originally described for splenectomy in sheep (18). Bilateral ovariectomy was performed via a single ventral midline incision, also as done previously in sheep (15).

Experimental designs:

Hyperoxia series: Six goats were hemorrhaged 0.5 ml/kg/min while exposed to normal air, 11% O_2 or, 100% O_2. Control measurements were obtained 60 minutes after cannulation, and then the inspired gas mixture was begun. After 30 min of breathing the prescribed gas mixture, the pre-

hemorrhage measurements were obtained. Hemorrhage was then begun, with measurements obtained at 10 min intervals up to 30 min. At this time the experiment was concluded and the hemorrhaged blood was returned.

Arginine vasopressin (AVP) infusion series: Six goats were infused with AVP at various doses while breathing either 11% or 21% O_2. A set of measurements was obtained 60 minutes after cannulation. The breathing gas was then initiated, and normal saline (vehicle) was infused for 20 min and the second set of measurements was obtained. The third through the seventh measurements were obtained following 20 min periods of AVP infusion at 30, 100, 300, 1000, 3000 pg/kg/min in that order.

Splenectomy series: Six goats had blood volumes assessed by the Evan's Blue dye dilution technique before and after splenectomy. They also were hemorrhaged before and after splenectomy similar to the protocol described above in the "Hyperoxia series". Following the hemorrhage experiments in the spleen-intact condition, the surgery was performed and the second hemorrhage was conducted a minimum of 3 weeks later, and within 2 months of the first experiment.

Estrogen series: Six female goats were splenectomized and bilaterally ovariectomized. Three neutered male goats were also used. Two sets of experiments were conducted on all goats with or without preceding daily subcutaneous injections of estradiol-17β (100 μg/kg). In the first set of experiments, conducted either without previous estrogen or following 2 weeks of daily estrogen, the goats were hemorrhaged at a rate 0.5 $ml \cdot kg^{-1} \cdot min^{-1}$. Prior to the hemorrhage and at 10 min, 20 min, and 30min or any period prior to 30 min when mean arterial blood pressure had fallen to 65 mmHg, the various cardiovascular parameters and blood samples were obtained. AVP, renin activity, and nor-epinephrine, hematocrit and osmolality measurements were obtained. Blood volume (Evan's Blue) was determined prior to each hemorrhage experiment in order to accurately determine the % blood volume loss required to lower blood pressure in the two states of circulating estrogen. Also, estradiol-17β was measured in the first blood sample to verify the effectiveness of the estrogen administration. In the second set of experiments the goats were infused with AVP (0.3, 10.0, 28.3 $ng \cdot kg^{-1} \cdot min^{-1}$), angiotensin II (1.25, 5, and 10 $ng \cdot kg^{-1} \cdot min^{-1}$), and phenylephrine (1.0, 1.8, and 6 $\mu g \cdot kg^{-1} \cdot min^{-1}$), each dose for 10 minutes with a one- to two-hour recovery in between different hormone infusions. The order of the hormone infusions was randomized. During each infusion, measurements of arterial blood pressure (BP), CO, CVP, PAP, and PAWP were recorded, and stroke volume and systemic vascular resistance (SVR) calculated. These experiments were also conducted without the presence of estrogen and also after 21 days of daily estrogen injections.

Statistics: There was a minimum of six goats in each series. The same goats were in each exposure and were sampled over several periods. Therefore, the Analysis of Variance (ANOVA) design used was a one-way with repeated measures. That is, the repeated measures were obtained over three experiments (for the data in figure 1) with 5 measurements in each experiment. Therefore these data were analyzed as a 6 (goat) by 15 (treatments) table. When the F value for the ANOVA was significant, the means of interest were compared by post hoc

analysis with a Fisher's LSD Multiple-Comparison test. The statistical software used was NCSS version 6.0.21 (Copyright 1996, Dr. Jerry L. Hintze, Kaysville, Utah).

KEY RESEARCH ACCOMPLISHMENTS:

Hyperoxia series:

As hypothesized, the fall in mean arterial pressure in response to 30 min of hemorrhage at 0.5 ml/kg/min was dependent upon the concentration of O_2 in the inspired air (Figure 1).

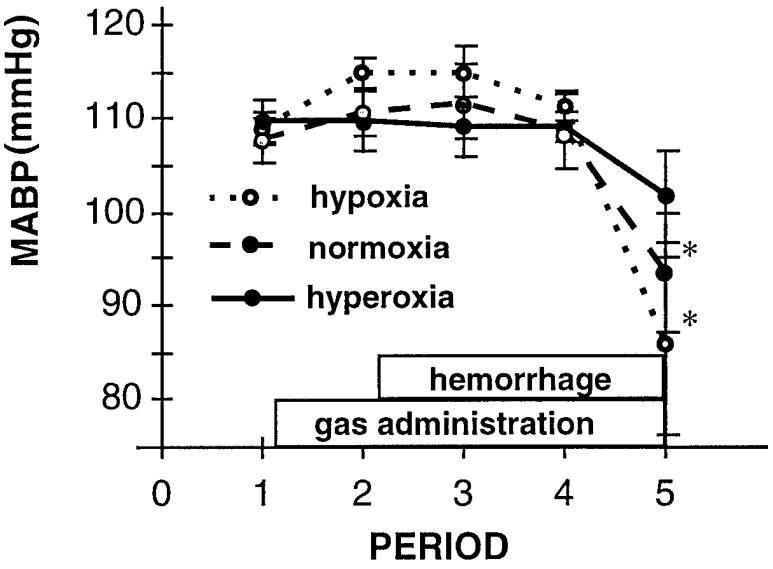

Figure 1. Response of mean arterial blood pressure to hemorrhage of 0.5 ml/kg/min. Period 1 = 60 min post cannulation, 2 = 30 min following initiation of either 11 %, 21% or 100% FIO_2, 3 = 10 min of hemorrhage, 4 = 20 min hemorrhage, 5 = 30 min hemorrhage. * = $P < 0.05$ compared to period 1.

It is noteworthy that with this magnitude of hemorrhage, 15/ml kg, inspiration of 100% O_2 prevented the fall in MABP, where both normoxic and hypoxic conditions were accompanied by significant decreases. Recent work by others appearing in an abstract has described a similar response in rats while breathing 100% O_2 following hemorrhage (19). Our work focused further on O_2 delivery and consumption. It was anticipated that 100% O_2 might improve the maintenance of arterial blood pressure, but at what consequence to O_2 distribution to the tissues? Decreased O_2 consumption (VO_2) may be an index of poor perfusion of tissues or perhaps the reduced work involved in breathing. Regardless, VO_2 did decrease with the initiation of 100% O_2 breathing (Figure 2). However, during hemorrhage the VO_2 returned

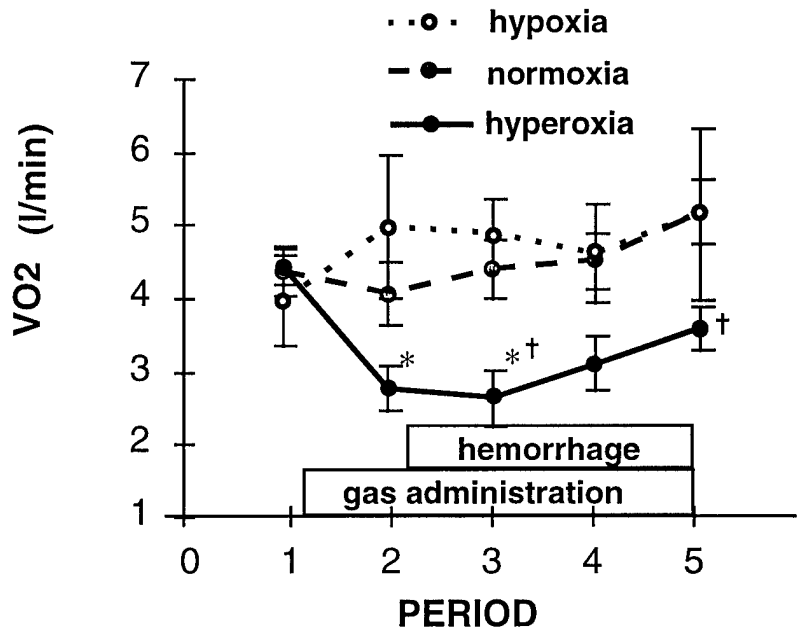

Figure 2. O_2 consumption (VO_2) during hemorrhage in conscious goat while breathing 11%, 21% or 100% O_2. The periods are the same as described for Fig.1., * = $P<0.05$ compared to period 1, † = $P < 0.05$ compared to normoxic conditions.

toward baseline, but VO_2 was still significantly below the values observed while breathing hypoxic or normoxic gases. Since the gap in VO_2 between hyperoxic and normoxic gas breathing appears to remain constant as VO_2 increases during hemorrhage, it is tempting to speculate that the cause for the reduction does not change. If this is the case, then hyperoxia does not compromise the necessary circulation to provide O_2 to those areas of demand during hemorrhage. Therefore, administration of 100% O_2 may be a safe and physiologically useful adjunct to emergency first aide to patients during hemorrhage, because of its ability to maintain perfusion pressure. Regional blood flow distribution studies will be necessary to assess the validity of this speculation.

We also hypothesized that, due to stimulation of chemoreceptors by hypoxia, that the vasopressin response to hemorrhage might be altered. Vasopressin was increased during the last period in all situations, but was significantly blunted during hyperoxia. This is expected because of the differences in MABP (Fig 1). However, subsequent regression analysis between MABP and plasma AVP indicated similar slopes with all breathing gas mixtures. In contrast, the low pressure system, as measured by right atrial pressure, was also correlated with plasma AVP levels, and hyperoxia was shown to significantly reduce the plasma AVP response to reduced stretch of the low pressure system baroreceptors. Vasopressin alters the regional distribution of blood flow, greatly favoring blood flow to the brain and heart, while shunting it away from the kidney, spleen, muscle, and skin (20). Thus, the reduced vasopressin response during hemorrhage during hyperoxia may work against its beneficial effects.

Clearly more work is needed to sort out the beneficial from potentially harmful effects of 100% O_2 administration during hemorrhage, but our results favor a beneficial effect.

Arginine vasopressin (AVP) infusion series:

The infusion of AVP into goats either while breathing hypoxic or normoxic gas mixtures resulted in nearly identical plasma levels of the hormone at all doses. Therefore, hypoxia resulted in no difference in AVP clearance contrary to observations made earlier on experiments in the neonatal piglet. However, exogenous administration of AVP did result in an increased PaO_2 (Figure 3). Note the significant improvement of PaO_2 at the highest infusion rate of AVP.

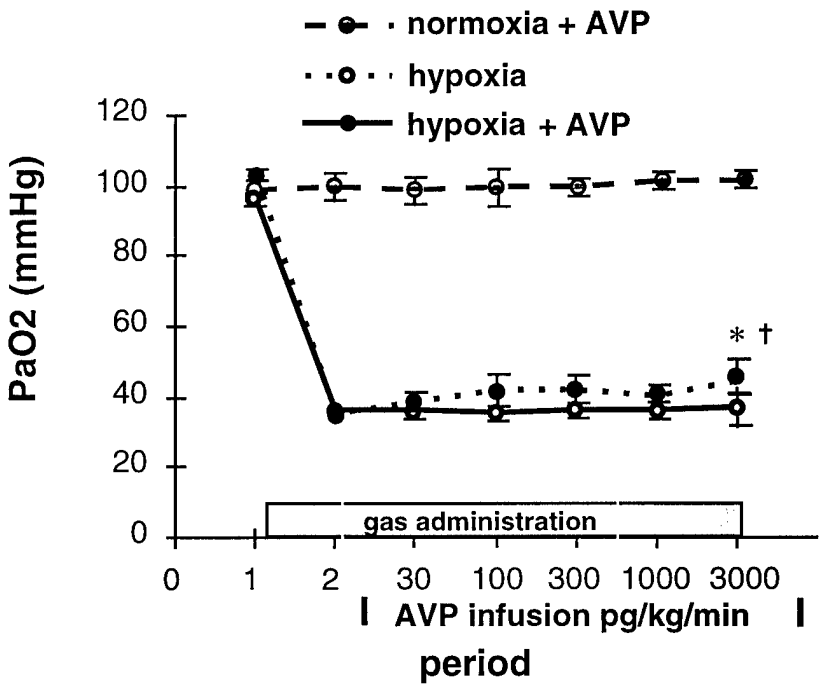

Figure 3. Effect of AVP infusion on PaO_2 during hypoxia and normoxia. Periods 1 and 2 are control periods 60 after catheterizations, and 30 min after initiation of 11% or 21% FIO_2 . AVP infusion rates are shown on the x axis, each infusion period was 20 min. * = $P<0.05$ compared to period 2. † = $P < 0.05$ compared to hypoxia without AVP infusion.

This improved PaO_2 was in association with a 10% improvement in arterial hemoglobin saturation ($P<0.05$), but because of the effects of AVP in decreasing cardiac output, O_2 delivery was not significantly improved. Never-the-less the effects of AVP on PaO_2 were confirmed in this model, albeit a modest response. In previous work in the anesthetized piglet model, we were able to infuse LVP (lysine vasopressin) at much higher doses (100 ng/kg/min), but these doses would not be tolerated in the conscious adult goat. Thus, the dose may have contributed to more obvious response we observed in the piglet. The present experiments, however, demonstrate that this response can be achieved by physiological levels of plasma AVP. In these experiments the plasma AVP levels were approximately 135 μU/ml in both infusion experiments which is common during hypotensive hemorrhage.

Splenectomy experiments:

Changes in MABP, cardiac output, and systemic vascular resistance were essentially identical when hemorrhage was performed before and after splenectomy. In addition, the hormone responses of vasopressin and renin were very similar. The most outstanding difference in the cardiovascular responses was the heart rate response shown below (Figure 4).

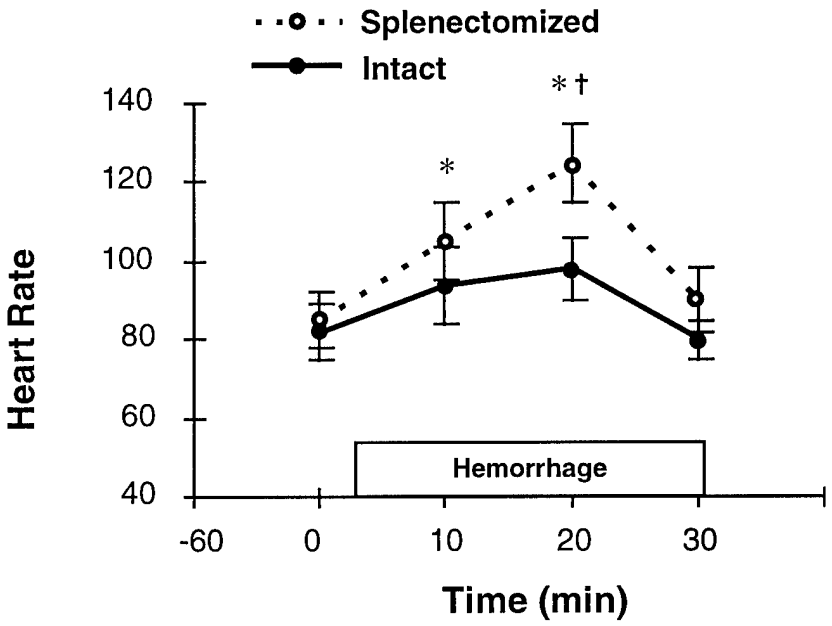

Figure 4. Effect of splenectomy on the heart rate response to hemorrhage (0.5 ml/kg/min). * = P<0.05 compared to pre-hemorrhage value. † = P < 0.05 compared intact condition.

Since the cardiac output was unchanged as a consequence of splenectomy, it would seem that stroke volume (SV) would have to be reduced in the splenectomized condition. In fact, mean values were lower, albiet not statistically significant, and this was due to a slightly reduced central venous pressure (CVP), also not significantly lower than in the intact state. Since hematocrit was significantly increased during the 20 and 30 minutes of hemorrhage, it would appear that the spleen was ejecting some volume of red blood cells into the circulation. This added volume may have contributed to the slightly improved CVP and SV during hemorrhage in the intact goat. Furthermore, it is likely that the inability to maintain SV in the splenectomized goat, reflexly resulted in increased sympathetic drive to maintain CO and adequate O_2 delivery. At this level of hemorrhage, in this model, the consequences of splenectomy on CO, O_2 delivery and consumption, and on hormonal responses to volume of hemorrhage was indiscernible.

Estrogen administration experiments:

These experiments are not quite finished, and many of hormonal analyses remain to be performed. However, some data are clear enough to draw conclusions at this point. In Figure 5 it can be seen that, as we hypothesized, the amount of blood loss required to cause a drop in

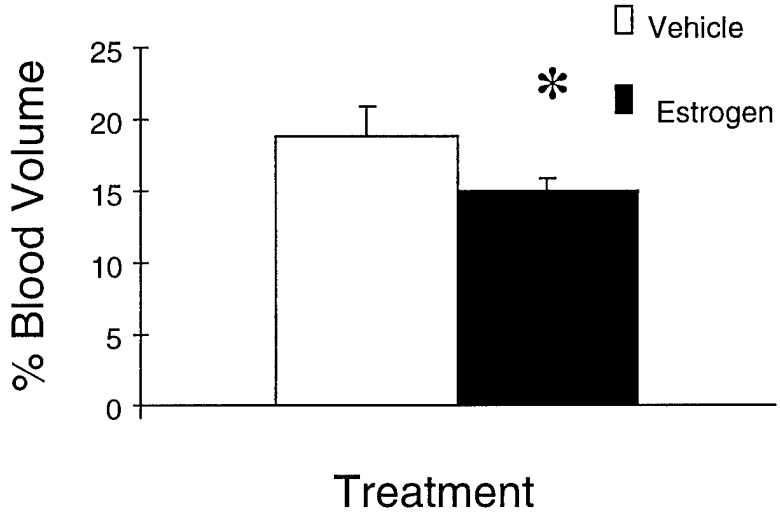

Figure 5. Effect of daily estradiol-17β administration to ovariectomized female goats (n = 6) on the blood volume loss required to induce a decrease in mean arterial pressure to 65 mmHg in response to hemorrhage (0.5 ml/kg/min). * = $P<0.05$ compared to saline injected control state.

mean arterial blood pressure to 65 mmHg, is significantly reduced when the goats have been given daily estrogen treatment for 2 weeks. The serum estrogen levels achieved by the daily subcutaneous injections were approximately 200 pg/ml, similar to the estrogen levels reported by Magness et al. (15) in studies in the sheep where they infused estradiol-17β intravenously for 2 weeks. Our data in 3 male goats revealed similar responses to the 6 female goats summarized in Fig 5. The apparent reason for this reduced tolerance to hemorrhage during estrogen administration, is not due to a reduced systemic vascular resistance as we had hypothesized, but rather a reduced cardiac output.

Furthermore, the data summarized in Figure 6 further support the lack of an effect of estrogen treatment on systemic vascular resistance. Note, even after 21 days of daily estrogen administration, the basal levels and hormone-stimulated values of systemic vascular resistance were not different between the control and the estrogen treated state. These findings are in contradiction to the findings of Magness et al. (15) and others, and we are as yet without a clear explanation. Several differences in the studies are evident; for example, the method of estrogen administration, the species, and the use of splenectomized animals in the present studies, but conjecture at this point is premature until all of the data has been analyzed.

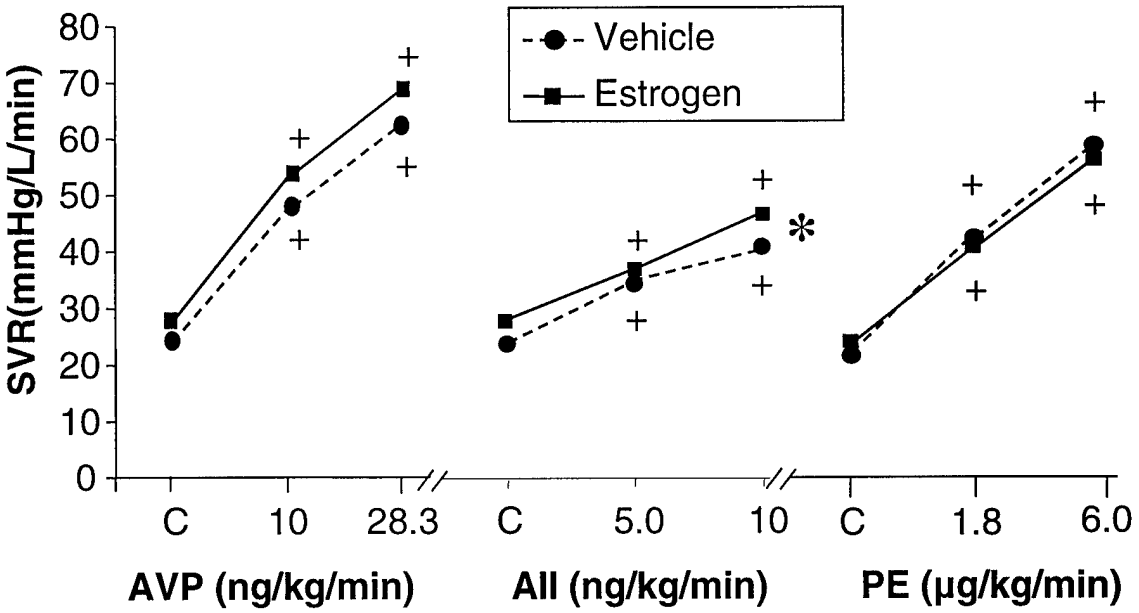

Figure 6. Effect of daily estradiol-17β administration for 3 weeks to ovariectomized female goats (n = 6) on the responses of systemic vascular resistance (SVR) to vasopressin (AVP), angiotensin II (AII) and phenylephrine (PE). * = $P<0.05$ compared to vehicle only at a similar time, + = $P<0.05$ compared to the control period (C) prior to hormone infusion.

REPORTABLE OUTCOMES:

Some of these studies have been presented in the following abstracts, and I anticipate that all of these will results in full manuscripts. In addition, the studies on the effects of estrogen on the hemorrhage response are nearly complete, but have not yet been presented, and these studies will result in a Ph.D.Thesis and they will be presented at the next Experimental Biology meetings in Orlando, Florida (2001). They too should result in a full manuscript.

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CONCLUSIONS:

The work in this project has produced the following findings: 1) Administration of 100 % O_2 during hemorrhage will help to maintain mean arterial blood pressure without compromising O_2 consumption. 2) Vasopressin improves PaO_2 during hypoxia, albiet very slightly, at physiological levels of plasma AVP. Also, hypoxia does not seem to alter the whole body clearance of AVP compared to the normoxic condition. 3) Splenectomy altered the responses to a slightly hypotensive hemorrhage in ways that lead to a clear difference in heart rate. Apparently owing to a reduced filling pressure, SV was not as well maintained as in the intact condition; however, CO was maintained by the increased heart rate. The mechanisms for this adjustment can not be determined from our data. 4) The presence of high levels of estrogen reduced the tolerance to hemorrhage in the conscious, splenectomized, ovariectomized goat.

Lastly, this grant has provided support to a graduate assistant, K.K. Urada for his pre-doctoral research, it provided considerable training experience for Dr. S.C. VanScoy, a neonatal fellow in our laboratory for 3 years. Mr Urada dedicated full time to the project for this past year with no stipend support for the last series of experiments that will be used for his Ph.D. Thesis to be defended at the end of summer. At the writing of this report there remain only two more experiments to be successfully executed before completion of the project. Manuscripts for the three completed studies are in various stages of preparation. The work developed in reference citation (3), and in the first abstract above, have resulted in an invitation for a Symposium on Sports Science at the '97 Winter Olympics in Nagano Japan this October, and the subsequent publication cited as reference (1).

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